Hemostaz Laboratuvarlarında Preanalitik Evre

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Preanalitik
Evre

Review

History of the preanalytical phase: a personal view

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Abstract

In the 70ies of the last century, ther term "preanalytical phase" was introduced in the literature. This term describes all actions and aspects of the "brain to brain circle" of the medical laboratory diagnostic procedure happening before the analytical phase. The author describes his personal experiences in the early seventies and the following history of increasing awareness of this phase as the main cause of "laboratory errors". This includes the definitions of influence and interference factors as well as the first publications in book, internet, CD-Rom and recent App form over the past 40 years. In addition, a short summary of previous developments as prerequesits of laboratory diagnostic actions is described from the middle age matula for urine collection to the blood collection tubes, anticoagulants and centrifuges. The short review gives a personal view on the possible causes of missing awareness of preanalytical causes of error and future aspects of new techniques in regulation of requests to introduction of quality assurance programs for preanalytical factors.

Key words: influence factors; interference factors; preanalytical factors; amylase in urine; haemolytic samples; anticoagulants; blood collection tubes; centrifugation

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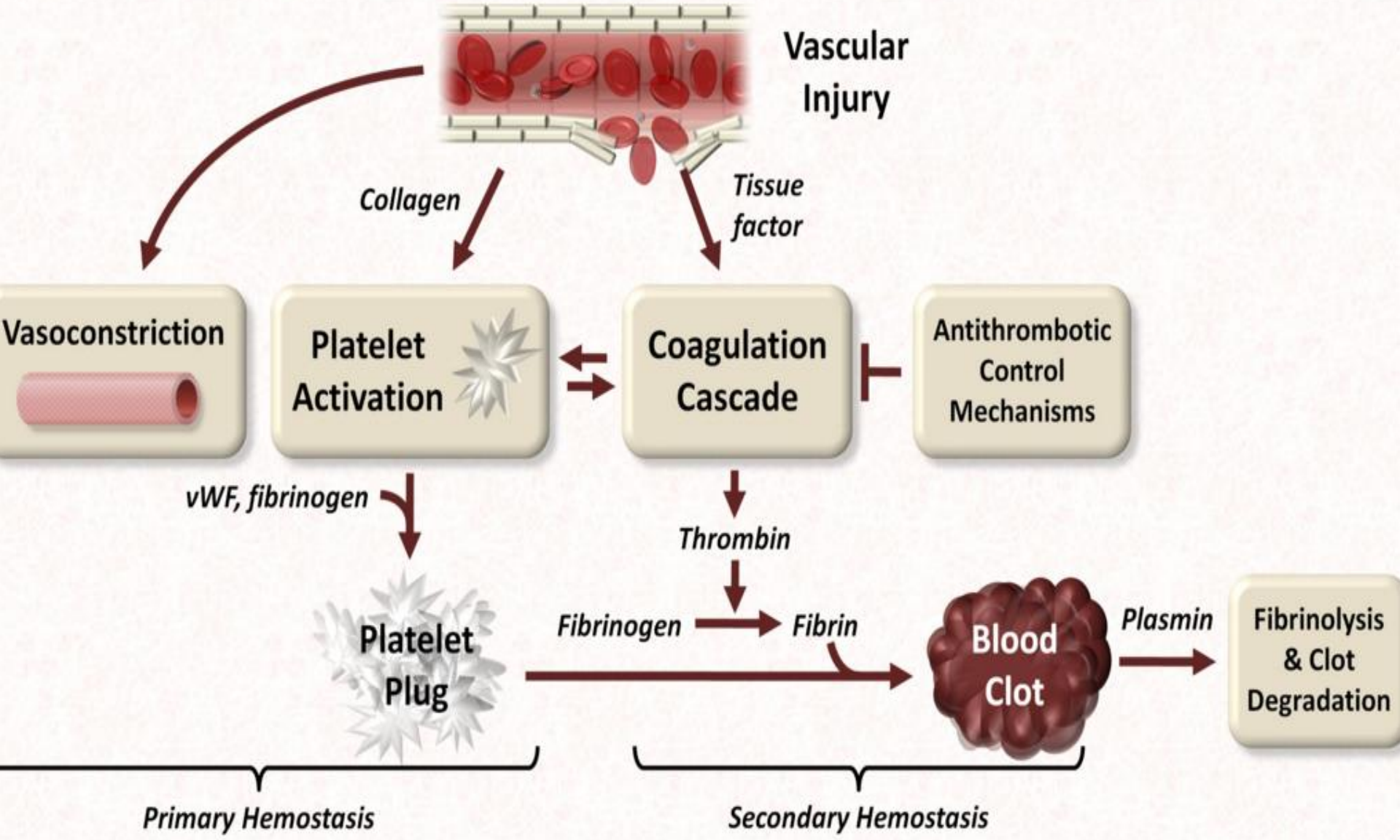
Major Components of Hemostasis



Primer Hemostaz Testleri:

*Kanama zamanı

*Trombosit fonksiyon testleri

Sekonder Hemostaz Testleri:

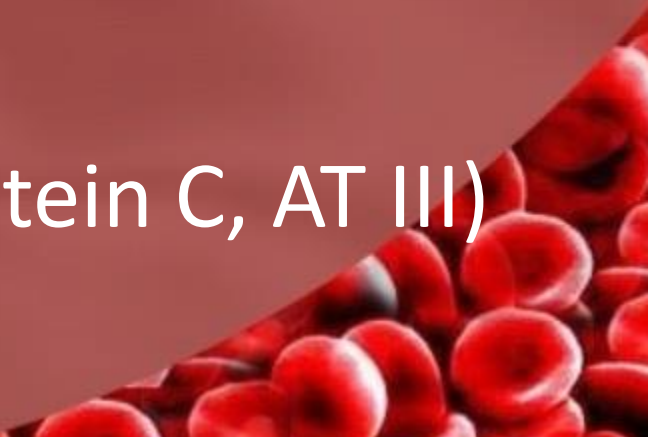
*Prothrombin Time (PT)

*Aktive Parsiyel Thromboplastin Time (aPTT)

*Trombin zamanı

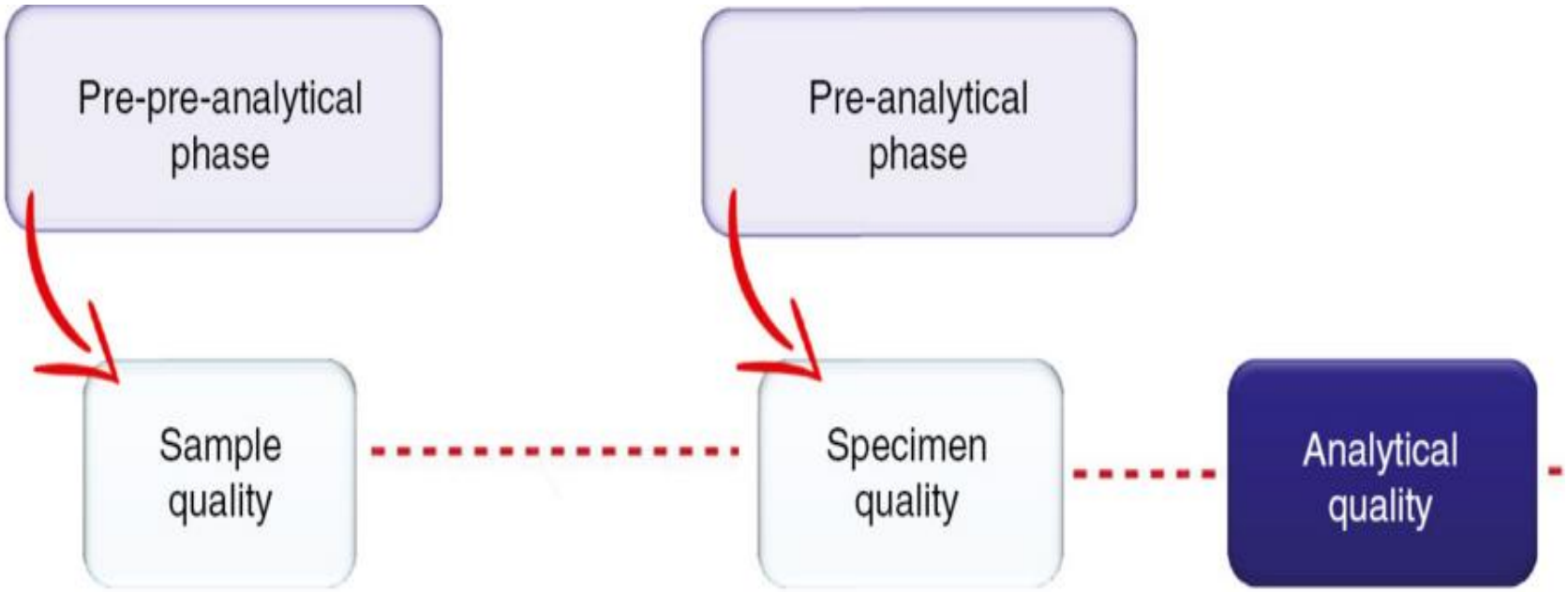
*Faktör düzeyleri (Faktör I-XIII)

*Tromboz paneli(Protein S, Protein C, AT III)



Hemostaz ve koagulasyon arasındaki farklar

Hemostaz ve Koagulasyon	
Hemostaz, vasküler hasarı takiben kanamayı durdurma sürecin tümüdür	Koagulasyon,hemostazın, trombositler ve çözünmeyen fibrin ağı tarafından stabil bir kan pıhtısının oluşturulduğu son aşamasıdır.
Prosesler	
Hemostazın nihai sonucu kanamanın durmasıdır.	Koagulasyon sırasında çözünebilir plazma fibrinojeni çözünmez fibrine polimerize olur ve yaralanmanın oluşturduğu boşluğu tıkanması için bir tıkaç oluşturur.
Tipleri	
Hemostaz, primer hemostaz ve sekonder hemostaz olarak adlandırılan iki tipe ayrılabilir.	Koagulasyon, kan pıtılaşmasının intrensek yolu ve ekstrensek yolu olarak kategorize edilebilir.
Hastalılar	
Hemostaz trombosit bozuklukları nedeniyle anormallikler gösterebilir.	Koagülasyon, karaciğer bozuklukları ve inaktif veya anormal fibrinojen üretimi ile bozulabilir.



The Five RIGHTS

- Right patient
- Right time
- Right test
- Right sample collection/handling
- Right sample transportation

The Five RIGHTS

- Right separation
- Right pre-treatment
- Right aliquoting
- Right sortation
- Right routing

Potential sources of preanalytical bias

Patient

Preparation
- Medication
- Hospitalisation
- Nutrition
- Fasting state

Use of stimulants
Pregnancy
Menstrual cycle
Genetic variants
Physical activity
Further diseases
Gut colonisation
etc.

Physician

Sampling mode
- Material
- Location
- Posture
- Disinfectant
- Time/Speed
- Tourniquet
- Ambience

Contamination
Staff differences
Storage at ward
Transport
etc.

Laboratory

Reception/unboxing
Storage conditions
- Temperature
- Duration
- Freezing/thawing
- Freezing speed

Ambience (eg. RT)
Preprocessing
- Centrifugation
- Pipetting

Analysis order
Batch processing
etc.

İki süper Derleme

Semin Thromb Hemost. 2008 Oct;34(7):612-34. doi: 10.1055/s-0028-1104540. Epub 2008 Dec 15.

Preanalytical and postanalytical variables: the leading causes of diagnostic error in hemostasis?

Favaloro EJ[1], Lippi G, Adcock DM.

Author information

Magnette *et al. Thrombosis Journal* (2016) 14:49
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Thrombosis Journal

REVIEW Open Access

Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories

A. Magnette[1], M. Chatelain[1], B. Chatelain[1], H. Ten Cate[2] and F. Mullier[1*]

1991-2016 PubMed taraması

Kan alınması konusunda anahtar preanalitik öneriler

- Silikonlu cam veya plastik (polipropilen) tüpleri kullanın
- **Kan örnekleri 105-109 mmol/Lsodyum sitrat, tamponlu antikoagülan içine çekilmelidir**
- **Antikoagüle edilen plazmanın pH'ı 7.3-7.45 arasında olmalıdır**
- Hastalarından kan toplama işlemini aç iken sabahları (7 ile 9 arasında) yapın
- Hasta rahatlatılmalıdır. Stresden kaçınılmalıdır.
- Kan alma tüpünü; hastanın tam adı, doğum tarihi, kimlik numarası ile etiketleyin.
- Venöz kanı, direkt olarak periferik bir damardan (antekubital ven) alın

- **İğnenin çapı tercihen 19 ve 22 gauge arasında olmalıdır**
- **İlk tüp dolmaya başladığında (<1 dk) turnikeyi serbest bırakın**
- **Flebotomi sırasında kan alma sırası, kan kültürü / steril tüpler, koagülasyon tüpleri, düz tüpler / jelli tüpler, katkı maddeleri içeren tüpler olmalıdır**
- **Sitratlı plazma kelebek sistemleri veya diğer IV kateteri kullanılarak elde edildiğinde alınan ilk tüpü kullanmayın**
- **Pıhtılaşma testleri için numune tüplerinin doğru doldurulmasını sağlamak için ilk atılan tüp değerlendirilebilir**
- **Tüplerin doğru doldurulduğundan emin olun (>% 90 dolum)**
- **Sodyum sitrat , gerekli tam kan oranına (1: 9) itibar gösterin**

Öneriler

- Fransız Haemostasis ve Tromboz Çalışma Grubu (GFHT),
- Dünya Sağlık Örgütü (WHO),
- Klinik ve Laboratuvar Standartları Enstitüsü (CLSI) kılavuzları,
- Uluslararası Tromboz ve Hemostasis Derneği (ISTH),
- Avrupa Klinik Kimya ve Laboratuvar Tıbbı Federasyonu (EFLM) ve
- İngiliz Hematoloji Standartları Komitesi (BCSH)
- ön analitik aşama ve preanalitik değişkenler hakkında sunmuş oldukları öneriler.

Örneğin işlenmesi konusunda anahtar preanalitik öneriler

- **Antikoagülanın tam dağılımını sağlamak için tüpler 3 ila 6 kere tam uçtan uca ters yüz ile yeterli ve hızlı bir şekilde karıştırılmalıdır**
- **Kan örneklerinin şiddetli çalkalanması, vortekslenmesi veya çalkalanmasını önleyin**
- **Hemotokritin yüksek olması durumunda sitrat hacmini ayarlayın (kan alımından önce örnekleme tüpünden sitrat çözeltisinin bir kısmını çıkar)**
- **Pıhtı veya hemoliz varlığını kontrol edin**

Örneğin transportu konusunda anahtar preanalitik öneriler

- Taşıma öncesinde, örnekleri, tanımlama, güvenlik koşulları ve stabilite ile ilgili olarak test edin
- **Örnekleri ortam sıcaklığında (15–25 ° C) mümkün olduğu kadar kısa bir sürede ve dik olarak nakledin. Trombosit fonksiyon analizi için pnömatik tüp sistemleri (PTS) kullanmayın**
- Örnekleri doğrudan laboratuvarda alın
- Alındıktan hemen sonra, tam kan taşıma sırasında kapaklı kalmalıdır
- Numunelerin analiz için tutulduğu odalarda sıcaklık kontrolü tavsiye edilir

Örneğin red edilmesi konusunda anahtar preanalitik öneriler

- Preanalitik işleme ve yerine getirilmemiş ulaşım gereksinimleri nedeniyle kabul edilemez olan tüm örnekler reddedilmelidir
- **Uygun olmayan toplama tüpleri, katkı maddeleri ve tarihi geçmiş tüpler**
- Hastanın tanımlanmasında hata veya eksiklik
- **Yetersiz hacim (analize bağlı olarak). Bir alternatif, ilave dilüsyona bağlı olarak sonucu adapte etmek olabilir (örneğin, faktör analizleri için, fibrinojen)**
- **Hemolizli (analize bağlı olarak) ve pıhtılı örnekler**

Santrifügasyon konusunda anahtar preanalitik öneriler

- **Rutin koagülasyon analizleri için, sıcaklık kontrollü santrifüj kullanın**
- **Trombosit-fakir plazma (PPP) elde edilir olmasını sağlamak için santrifüjü kullanmadan önce, her 6 ayda bir veya modifikasyonlardan sonra doğrulayın**
- **Santrifüj bakımının yapılmaması nedeniyle titreşim olmadığını (hızlanma/ yavaşlama işlemleri sırasında) kontrol edin**
- **Pıhtılaşma testi için primer tüpü 1500g, 15 dk'da santrifüj edin. Acil durumlarda, taze plazmada PTT, APTT ve fibrinojen için yüksek santrifüj gücü (1500 g'dan büyük) ve daha kısa süre (10 dakikadan az) kullanılabilir**
- **PPP'nin hazırlanması, $10x10^9$/L'den daha düşük bir rezidüel trombosit sayısı elde etmek için çift santrifüjleme gerektirir.**İlk santrifüjlemeyi takiben, otomatik bir pipet kullanarak plazmayı aktif olmayan bir plastik santrifüj tüpüne dikkatlice aktarın ve daha sonra yaklaşık 15 dakika boyunca tekrar santrifüjleyin
- Trombosit fonksiyon analizi için trombosit açısından zengin plazmanın (PRP) hazırlanması,bir rotor freni uygulanmadan 10 dakika boyunca 200–250 g santrifuj gerektirir.

Örneğin Depolanması konusunda anahtar preanalitik öneriler

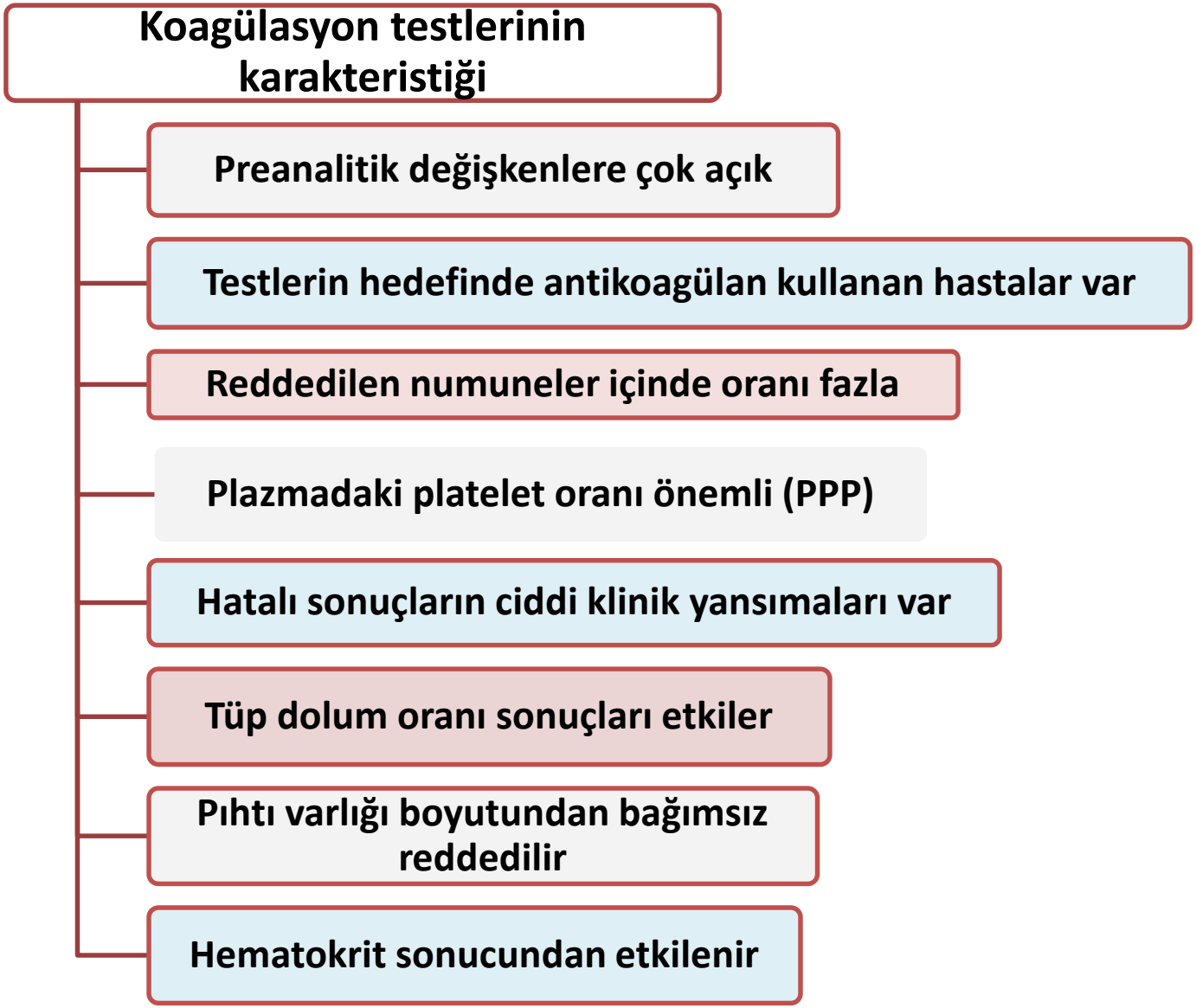
- **Numuneleri analize kadar oda sıcaklığında (15–25 ° C) saklayın**
- **Tam kan analizlerini kan alınımından <4 ve santrifüj <1 saat sonra gerçekleştirin**
- Aşırı sıcaklık dereceleri (örneğin hem soğutulmuş hem de yüksek) önlenmelidir
- PPP'yi oda sıcaklığında (15–25 ° C) veya analize kadar −80 ° C'de saklayın
- **Tam kan örneği toplamadan 1 saat sonra santrifüj edilirse, plazma test edilmeden önce 4 saate kadar oda sıcaklığında bırakılabilir**
- **Örnek alımından analize kadar geçen zaman analite bağlıdır: PT / INR için örnekler oda sıcaklığında daha uzun bir stabiliteye sahiptir (24 saat)**

- **Trombosit fonksiyon testleri için, numuneler analizden en az 15 dakika önce oda sıcaklığında bekletilmelidir. Test örnek alımından<3–4 saat içinde tamamlanmalıdır**
- Faktör V ve VII analizi <3 saat yapılmalıdır
- **Fibrinojen, protein C ve antitrombin aktivitesi 7 güne kadar oda sıcaklığında saklandığında nispeten sabit kalır**
- **Protein S aktivitesi kararsızdır ve 8 saatte istatistiksel olarak anlamlı bir aktivite kaybı vardır**
- **VWF, oda sıcaklığında 48 saat boyunca sabit gibi görünmektedir**
- **APTT için örnekler taze plazma <4 saat (fraksiyone olmayan heparin ile tedavi edilen hastalarda <1 saat) kullanılarak yapılmalıdır**

Örneğin dondurma ve çözdürme konusunda anahtar preanalitik öneriler

- **Donmuş örneklerden PT, aPTT ve faktör VIII testlerini çalışmayın**
- **4 saat içinde test edilemeyen örnekleri santrifüjleyin ve plazma alikotunu dondurun**
- Hızlı dondurma tekniğini (sıvı azot) kullanın
- Örnekleri −20 ° C yerine −70 ° C'de (veya altında) saklayın
- **-20 ° C'de dondurulmuş plazma örnekleri 2 hafta, - 80 ° C'de dondurulmuş plazma 6 - 18 ay boyunca parametreye bağlı olarak stabil kalır**
- **Örnekleri tekrar dondurmayın (ancak yeterli sayıda alikot hazırlayın)**

- **Numuneleri hızlı bir şekilde 37 ° C'de (fibrinojen denatürasyonunu önlemek için) bir su banyosunda 37 ° C' de en az 5 dakika boyunca, (oda sıcaklığında değil),bir tezgah üzeinde veya bir mikrodalga fırında içinde çözün. Hemen test edin**
- Çözüldükten sonra, herhangi bir cryoprecipitatı resüspanse etmek için numuneyi hafifçe karıştırın. Vortekslemeyin veya sallamayın


Koagülasyon testlerinin karakteristiği
Preanalitik değişkenlere çok açık
Testlerin hedefinde antikoagülan kullanan hastalar var
Reddedilen numuneler içinde oranı fazla
Plazmadaki platelet oranı önemli (PPP)
Hatalı sonuçların ciddi klinik yansımaları var
Tüp dolum oranı sonuçları etkiler
Pıhtı varlığı boyutundan bağımsız reddedilir
Hematokrit sonucundan etkilenir

Koagulasyon Testlerinin Karakteristiği

- 1-Preanalitik değişkenlere çok açık
- 2- Testlerin hedefinde Antikoagulan ilaç kullanan hastalar var
- 3-Red edilen numuneler içinde oranı fazla
- 4-plazmadaki platelet oranı önemli(PPP)
- 5-Sonuç hataların ciddi klinik yansımaları var
- 6-tüp dolum oranı sonuçları etkiler
- 7-Pıhtı varlığı boyotundan bağımsız red nedenidir
- 8-Hemotokrit sonucundan etkilenir

Table 1_Summary of Hemostasis Tests and Sample Requirements

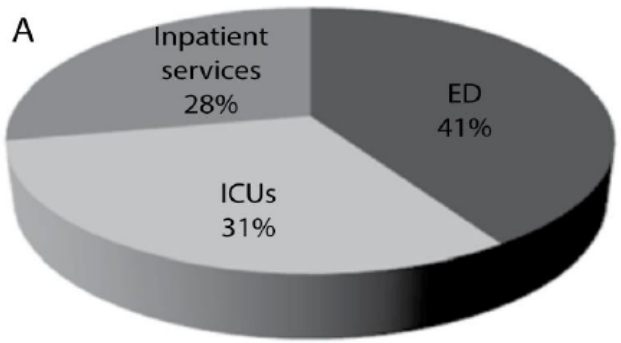
Comprise	Usually Performed Via	Sample Type
A. *Routine coagulation tests*		Citrate anticoagulated plasma post single centrifugation
PT/INR, APTT, TT, fibrinogen	Clot-based tests, automated instrument, primary collection tube (sometimes separated plasma)	
D-Dimer (D-D)	ELISA or ELFA or agglutination (primary or secondary tube)	
B. *Specialized Hemostasis Tests*		
Factor assays (ie, II, V, VII, VIII, IX, X, XI, XII), factor inhibitor assessments, protein S, protein C	Clot-based tests, automated instrument	Separated citrate anticoagulated plasma, post single centrifugation (usually post freezing)
VWF tests	ELISA, immunoassay, or agglutination	
Protein C, protein S, antithrombin	ELISA, immunoassay, clot based, or chromogenic assays	
Heparin (anti-Xa) assay	Chromogenic assays	Separated citrate anticoagulated plasma, post single (or preferably double) centrifugation (usually post freezing)
APCR	Clot-based tests, automated instrument	
LA	Clot-based tests, automated instrument	Separated citrate anticoagulated plasma, post double centrifugation (usually post freezing)
Solid phase aPL tests including aCL and ab2GPI	ELISA or immunoflourescent assay	Separated serum preferred; separated citrate anticoagulated plasma post single centrifugation sometimes acceptable. Usually post freezing.
Platelet function tests	Specialized instrumentation	Citrate anticoagulated whole blood or special processing required.
Genetic thrombophilia tests	Specialized instrumentation	EDTA or citrate anticoagulated whole blood or special processing required

Hata nedenine Bağlı Örnek reddi

Laboratory test groups	Improperly labelled samples (%)	Hemolysis (%)	Clotted specimen (%)	Insufficient volume (%)
Clinical chemistry inpatient	0.03*	0.06	0	0.01
Clinical chemistry outpatient	0	0	-	0
Immunoassay	0	0	-	0.10
Coagulation	0.02	0.48	0.26	1.38**
HbA1c	0.01	-	0.01	0.01
Hematology	0.01	-	0.16*	0.02
ESR	0.01	-	0.64*	0.25

Biochemia Medica 2014;24(3):376–82

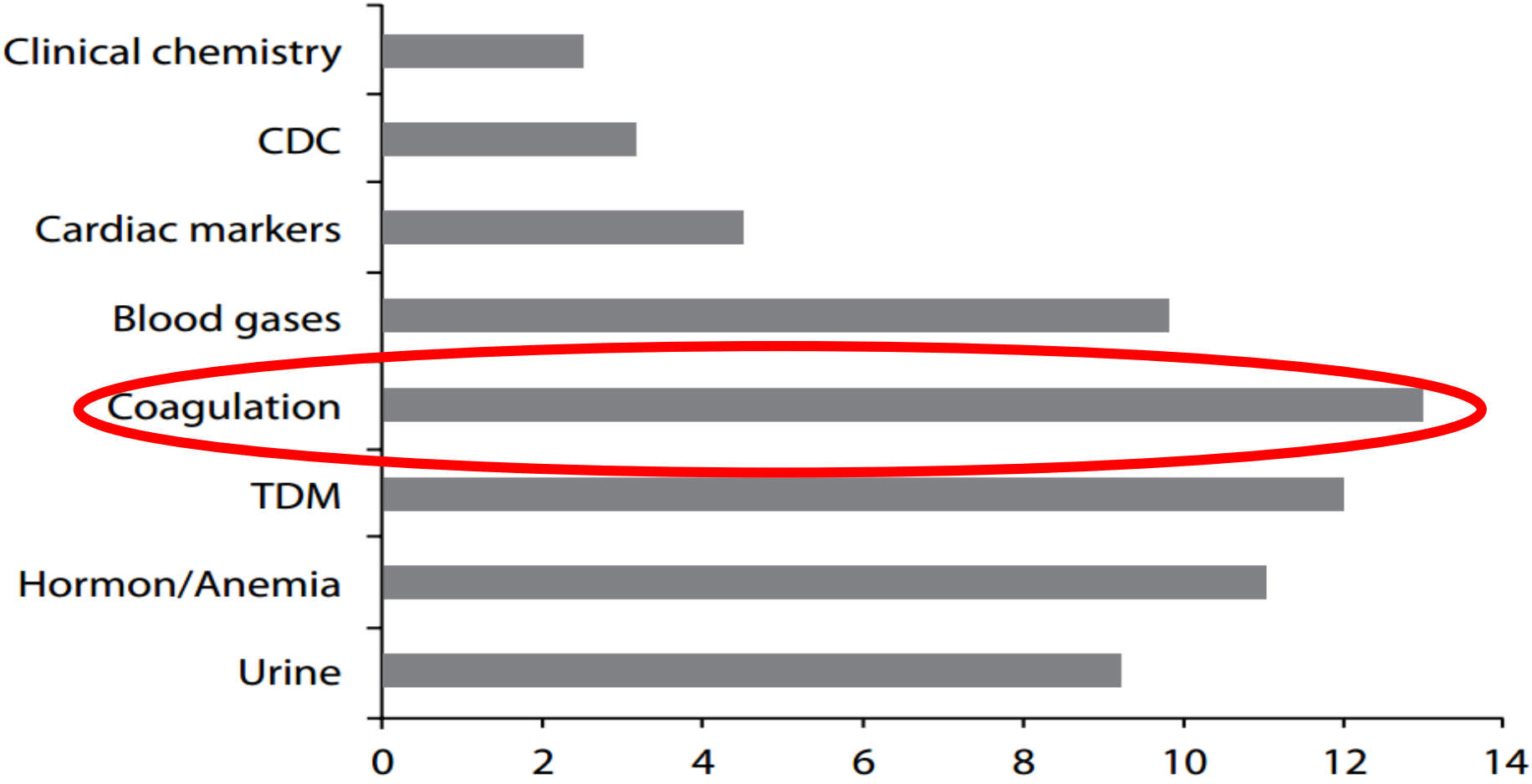
Örnek Kabulünde Red Nedenleri



Pıhtılı örnek	%38.4
Uygunsuz örnek hacmi	%33.2
Hatalı klinik istem	%7.3
Hatalı kimlik tanımlama	%6.5
Hemoliz	%6
Yanlış tüp	%3.8
Gecikmiş transport	%1.5

Z.G. Dikmen, A .Pınar, F. Akbıyık. Specimen rejection in laboratory medicine: Necessary for patient safety? Biochemia Medica 2015;25(3):377–85

Acil Örneklerde Red Yüzdesi



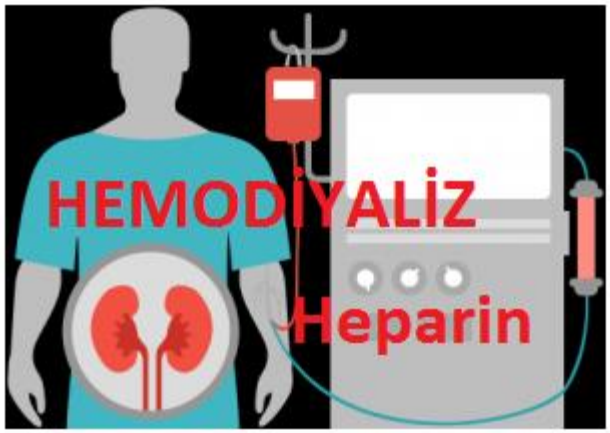
Biochemia Medica 2015;25(3):377–85

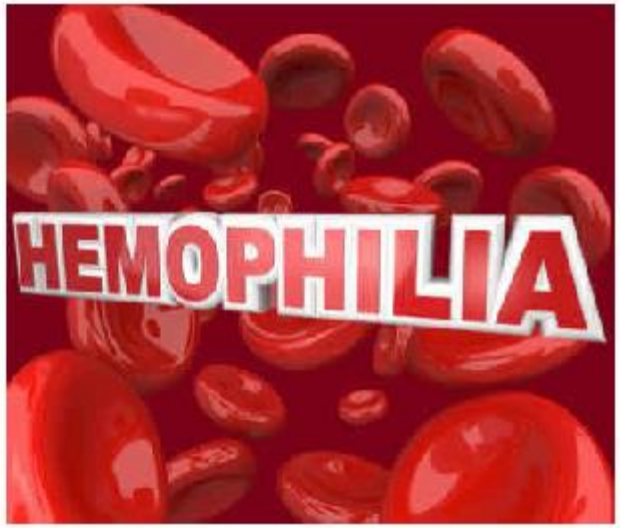

HATALI
NUMUNELER
ATILMASIN


HATALI
UYGULAMALAR
OLUŞMASIN

?


KOAGÜLOMETRE
Coagulometer


HEMODİYALİZ
Heparin


HEMOPHILIA

Hemotokrit değeri/sitrat oranı

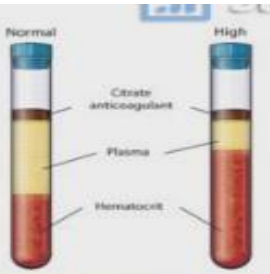
X = (100–PCV)*vol./(595–PCV)

Where:
X= volume of sodium citrate
Vol =volume of whole blood drawn
PCV= patient's hematocrit

Examples:

Patients Hct= 60%, V= 5 mL
X=(100-60)*5 / (595-60)
= 40*5 / 535 = 0.34 ml

Patient Hct = 25%, V=5 ml
X=(100-25)*5 / (595-25)
= 75*5 / 570 = 0.65 ml



HCT	Citrate (ml)
0.20	0.70
0.25	0.65
0.30	0.61
0.55	0.39
0.60	0.36
0.65	0.31
0.70	0.27

Platelet Poor Plasma (CLSI H21-A5)

Assay	RT	Refrig.	-20°C	-70 °C
PT	Up to 24 hrs	Unacceptable	2 weeks	12 mo.
APTT	4 hrs	4 hrs	2 weeks	12 mo.
APTT (UFH)	4 hrs	4 hrs	2 weeks	Unknown
APTT (VWF, FVIII)	4 hrs	4 hrs	2 weeks	6 mo.
Other	4 hrs	4 hrs	Analyte-dependent	

Preanalytical Interferences Associated with Diagnostic Errors[2, 5,]

	Hemolysis	Icterus	Lipemia	Improper Tube Filling
Falsely increased results	PT, D-dimer, FV, FVII, FX	PT, Fibrinogen	Antithrombin	PT, APTT
Unaffected results		APTT	PT, APTT	
Falsely decreased results	APTT, Fibrinogen, Antithrombin, Thrombin Time	Antithrombin	Fibrinogen	D-dimer

2. Favaloro EJ, Lippi G, Funk (Adcock) D. Labmedicine. 2012 Feb;43(2).

5. Lippi G, Plebani M, Favaloro EJ. Semin Thromb Hemost. 2013;39:258–266.

ble 2 Summary of main results for hemostasis tests

	1,500-S	3,000-S	*p*	1,000-S	*p*	500-S	*p*
	Value	Value		Value		Value	
APTT (s)							
Values	30.6 (28.8–32.3)	30.0 (28.6–31.5)	0.35	30.2 (29.3–32.1)	0.87	30.2 (28.2–32.0)	0.39
Bias	–	−0.4 (−1.2 to 0.5)		−0.1 (−0.8 to 0.7)		−0.3 (−1.1 to 0.5)	
PT (s)							
Values	10.7 (10.2–10.9)	10.5 (10.0–11.0)	0.08	10.4 (9.7–10.8)	0.01	10.3 (9.9–10.7)	< 0.01
Bias	–	−0.2 (−0.4 to 0.0)		−0.4 (−0.6 to −0.1)		−0.4 (−0.6 to −0.2)	
FBG (g/L)							
Values	277 (249–325)	269 (246–360)	0.25	299 (284–326)	0.01	302 (284–332)	< 0.01
Bias		12 (−9.5 to 33)		23 (8–38)		27 (15–40)	
FVIII:C (U/dL)							
Values	65 (61–85)	76 (65–98)	0.04	72 (61–90)	0.15	73 (61–100)	0.08
Bias		14 (1–27)		5 (−2 to 13)		9 (−1 to 19)	
FVIII:A (U/dL)							
Values	60 (52–76)	70 (58–84)	0.03	57 (50–76)	0.86	62 (44–84)	0.43
Bias		11 (1–22)		0.7 (−8 to 9)		4 (−6 to 14)	
FIX:C (U/dL)							
Values	82 (70–110)	99 (86–115)	0.01	98 (85–115)	< 0.01	103 (83–121)	< 0.01
Bias		10 (5–15)		10 (5–14)		12 (8 to 16)	

Table 1. Effect of various anticoagulants on routine and select specialty coagulation assays

Assay	UFH	LMWH fondaparinux	VKA	Dabigatran (thrombin inhibitor)	Rivaroxaban or apixaban (Xa inhibitors)
APTT	Prolonged ↑ ↑	No effect or prolonged ↑	Prolonged ↑	Prolonged ↑ ↑	Prolonged ↑
PT/INR*	Little or no effect	No effect	Prolonged ↑ ↑	Prolonged ↑	Prolonged ↑ ↑
TCT	Prolonged ↑ ↑ ↑	Prolonged ↑	No effect	Prolonged ↑ ↑ ↑	No effect
Clauss fibrinogen	May be factitiously low	No effect	No effect	No effect or factitiously low†	No effect
AT activity					
a. FXa based	a. & b. may be decreased‡	a. & b. No effect	a. & b. No effect	a. No effect	a. Factitiously overestimated
b. FIIa based				b. Factitiously overestimated	b. No effect
PC activity					
a. Clot based	a. Factitiously overestimated	a. & b. No effect	a. & b. Decreased‡	a. Factitiously overestimated	a. Factitiously overestimated
b. Chromogenic	b. No effect			b. No effect	b. No effect
PS activity					
a. Clot based	a. Factitiously overestimated	a. & b. No effect	a. & b. Decreased‡	a. Factitiously overestimated	a. Factitiously overestimated
b. Free PS Ag	b. No effect			b. No effect	b. No effect
APTT-based APCR with added FV deficient plasma	Factitiously elevated ratio	No effect	Factitiously elevated or decreased ratio possible	Factitiously elevated ratio	Factitiously elevated ratio
APTT-based factor assays, one stage	Factitiously low FVIII, IX, XI	Factitiously low FVIII, IX, XI§	Decreased FIX‡	Factitiously low FVIII, IX, XI	Factitiously low FVIII, IX, XI§¶
PT- based factor assays, one stage*	No effect	No effect	Decreased FVII, X, II‡	Factitiously low FII, V, VII, X§	Factitiously low FVII, X, V, II¶
Chromogenic FVIII activity	No effect	No effect	No effect	No effect	Factitiously low
APTT mixing study	Incomplete correction	Incomplete correction	Correction into normal range	Incomplete correction	Incomplete correction¶
PT mixing study*	Not indicated with normal PT	Not indicated with normal PT	Incomplete correction	Incomplete correction	Incomplete correction¶
LA tests	Possible to misclassify as LA	Effect unlikely	Possible to misclassify as LA	Possible to misclassify as LA	Possible to misclassify as LA¶

	Desirable bias	EDTA contamination				
		0%	5%	13%	29%	43%
APTT						
Values (s)		30.7 ± 1.4	30.4 ± 1.3	30.6 ± 1.6	32.9 ± 1.6*	37.3 ± 2.0*
Bias (%)	±2.3%	–	−1% (−2 to 0%)	0% (−1 to 1%)	7% (6 to 9%)	22% (19 to 24%)
PT						
Values (s)		10.9 ± 0.4	11.0 ± 0.5	11.0 ± 0.5	11.6 ± 0.6*	13.8 ± 0.9*
Bias (%)	±2.0%	–	0% (0 to 1%)	1% (0 to 2%)	6% (5 to 8%)	26% (23 to 29%)
Fibrinogen						
Values (g/L)		2.9 ± 0.6	2.9 ± 0.6	2.9 ± 0.6	2.8 ± 0.6	2.3 ± 0.5*
Bias (%)	±4.8%	–	−1% (−4 to 1%)	−1% (−5 to 2%)	−3% (−7 to 1%)	−21% (−15 to −27%)

*$P < 0.001$ compared to the uncontaminated aliquots.

Table 3_Summary of Effects of Inappropriate Sample Processing Issues on Select Hemostasis Tests

Issue	Effect on Hemostasis Tests
Whole blood refrigerated prior to centrifugation	Platelet activation and loss of FVIII and VWF; can lead to false diagnosis of hemophilia or VWD
Filtered plasma	Loss of fibrinogen, FVIII, and VWF; can lead to false diagnosis of dysfibrinogenemia, hypofibrinogenemia, hemophilia, or VWD; prolongs routine coagulation test times (PT, APTT, and TT); false LA feasible
Delayed transport, delayed testing, poor storage, several freeze-thaw events; storage in frost-free freezer	1. Loss of labile factors (especially FV and FVIII); can lead to false impression of hemophilia; prolongs routine coagulation test times (PT, APTT); 2. Samples with unfractionated heparin can yield lower than expected APTTs and lower anti-FXa (heparin) test levels; 3. Potential activation of FVII
Poor centrifugation, heavy braking, sample remixing prior to freezing	Platelet contamination, hemolysis, and platelet disruption post freezing; activation, false low APTT, false low heparin levels, false negative LA, false high factor levels

Table has been adapted and updated from reference 4.

Pre-analitik Değişken Flagları Standardize eden Otomasyon Teknolojileri

Technological Advances in the Hemostasis Laboratory

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Semin Thromb Hemost 2014;40:178–185.

Rekor 4.69 saniye

Koagülasyonda otomasyon

- Koagülasyon testi son 50 yılda dramatik bir değişim yaşadı. 1950'lerde bile, 1960'lar ve belki de 1970'in koagülasyon testleri elle yapıldı.

- Bir santrifüj, bir kronometre, bir pipet ve bir test tüpü ile donatılmış laboratuvar teknisyeni, bir kanca ile ince bir iğne kullanan bir hastanın numunesinin pıhtılaşma kapasitesini belirlerdi. Onun kararına göre, kancanın ucunda küçük bir fibröz pıhtı tespit edildiğinde saat durduruldu.

.

- Daha sonra, testleri standartlaştırmak ve optimize etmek için, küçük pıhtıların oluşumunu belgelemek için küçük pıhtının ışık geçirgenliği veya
- küçük boncukların pıhtının büyümesi ile hareket etmesinin engellenmesini dokümente eden ve **fotometre** mantığı ile çalışan küçük cihazlar üretilmiştir.

- Koagülasyon testleri, in vivo olarak meydana gelen olaylara benzer bir şekilde in vitro bir işlemi taklit etme avantajına sahiptir.
- Bununla birlikte, bu testler in vitro olarak hemoliz, ilaç ve lipidemia gibi birçok farklı etkiye yatkındır.

- Sysmex® CS-5100i (Siemens, Münih, Almanya) gibi yeni nesil koagülasyon sistemleri. hem preanalitik hem de yeni analitik özelliklere sahiptir.
- Preanalitik cihaz, 3 farklı dalga boyu (405 nm, 575 nm ve 660 nm) kullanarak hasta numunelerini tarayarak hemoliz, ikterus ve lipemiyi tespit eder.
- Bu özellik, analizden önce potansiyel olarak sorunlu test örneklerini tanımlamak için ekstra operatör desteği sunar.
- Hatalı örnek toplamadan kaynaklanan uygunsuzlukları hesaba katarak numune hacmi de kontrol edeler.

Evaluation of Coagulation tests:

- Manual Method:
- Coagulometers:
  - Semiautomated Method.
  - Automated Method.

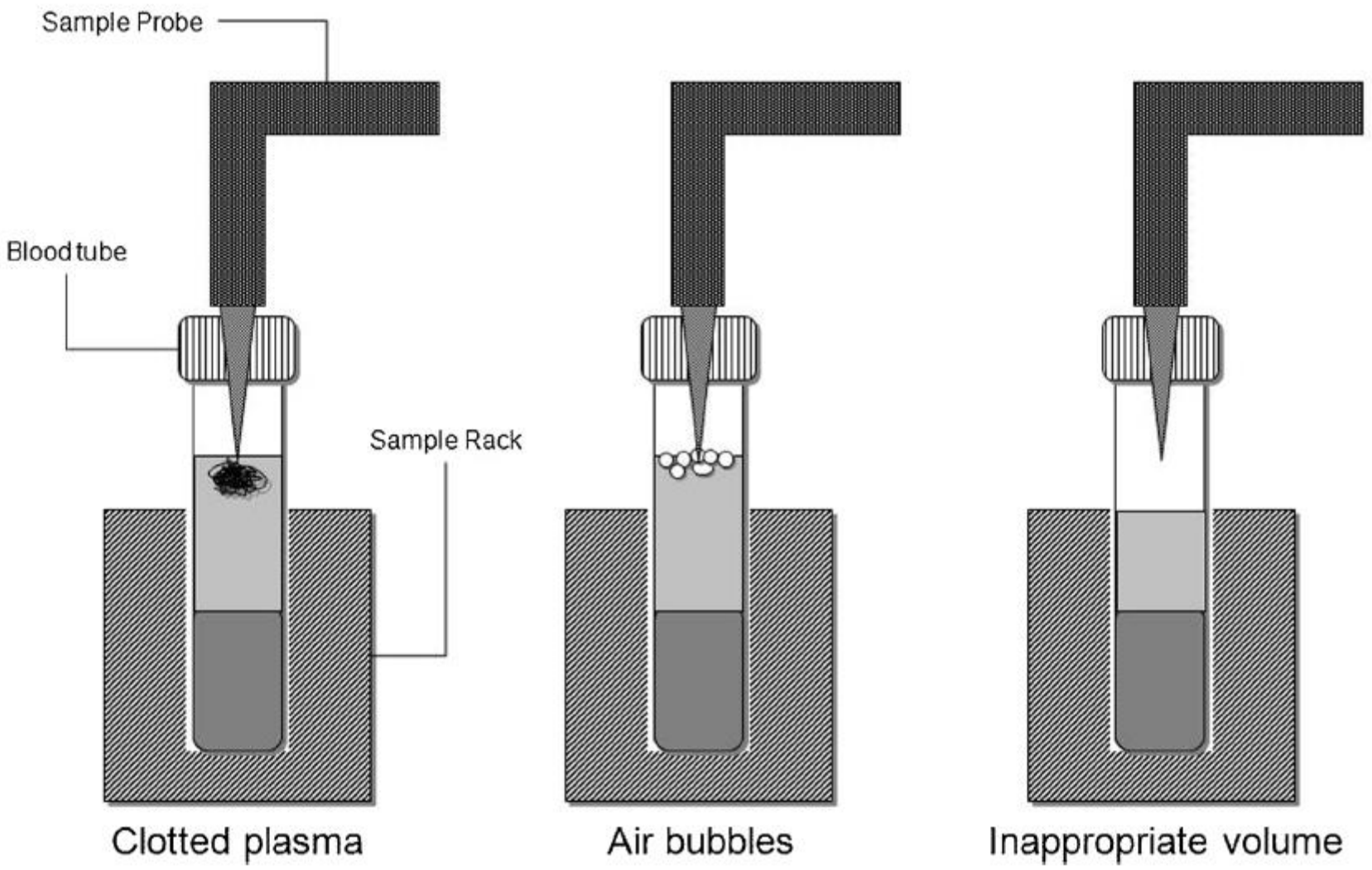
Methods of Endpoint Detection

- Mechanical
- Optical
  - Photo-optical
  - Nephelometric
  - Chromogenic
  - Immunologic
- Electrochemical

a

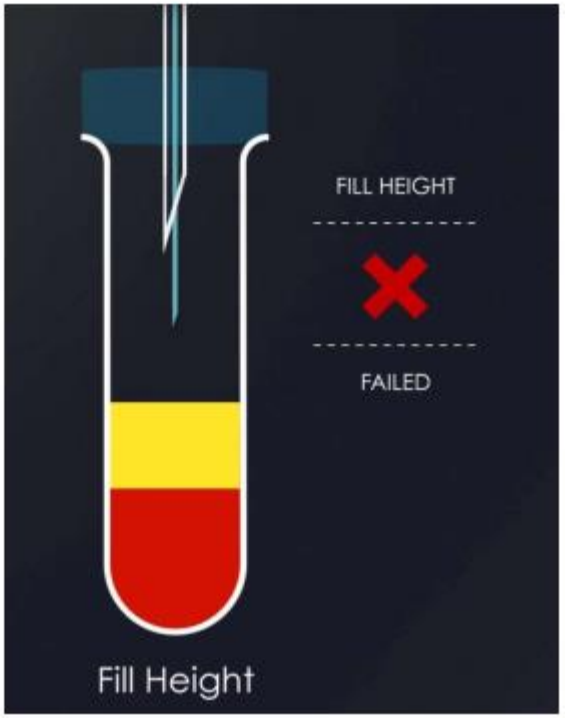
b

c


Sample Probe
Blood tube
Sample Rack
Clotted plasma
Air bubbles
Inappropriate volume

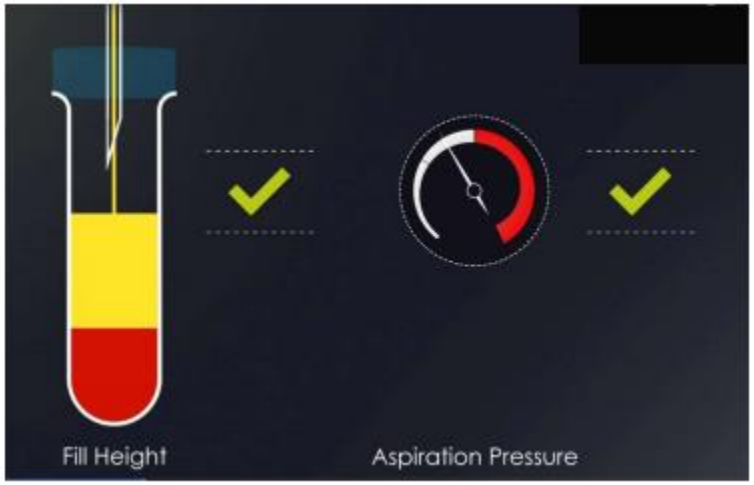
Automatic Detection of Under-filled Tubes

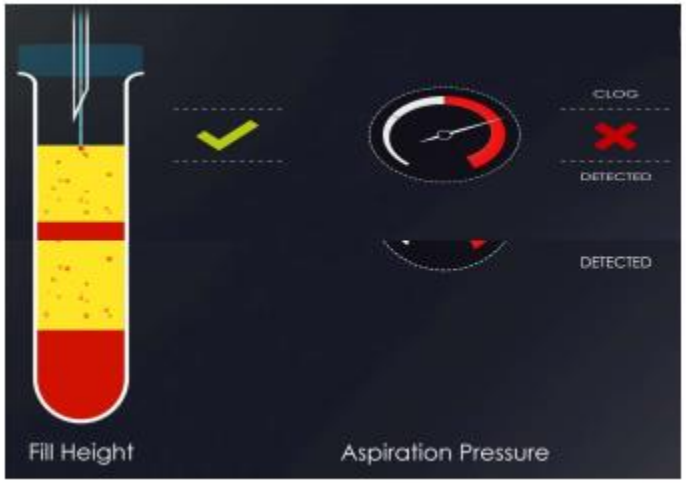
- Alerts operator to possible sample-collection errors
- Ensures appropriate sample:anticoagulant ratio
- Verifies sample-draw quality



Abnormal Sample Aspiration

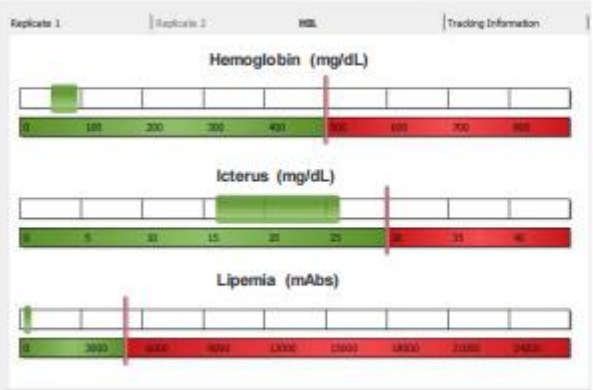
- Alerts operator to possible clogs in plasma



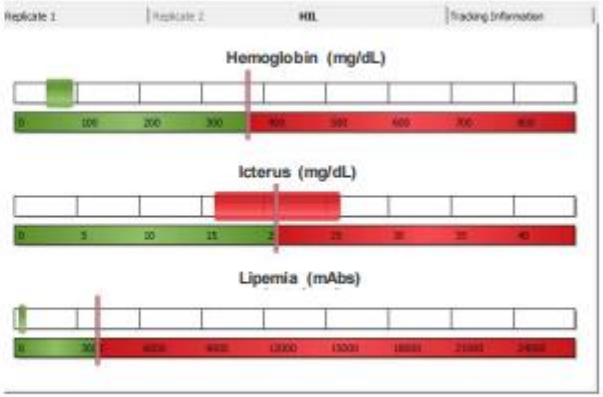


Assay-Specific Interfering Substance Detection
Hemolysis, Icterus and Lipemia

- Assay-specific thresholds are best
- Base thresholds on real test results
- Same interference substance readings can flag results for one assay, but not another



PT with no flag



Fibrinogen with flag

Evaluation of Plasma Index in Coagulation Testing on the Sysmex CS-5100: HIL Index

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[2]Pediatric Hematology, Erciyes University, Kayseri, Turkey

Background: An important part of medical decisions are based on the laboratory results. Coagulation testing is vital value in diagnostic approach in patients with hemostatic disturbances, and it is necessary for monitoring antithrombotic therapy. Variety of errors can occur in the preanalytical, analytical and postanalytical steps of the process. Preanalytical factors are the most common source of interference. Hemolysis, icterus and lipemia (HIL) in specimen may affect the accuracy of coagulation test results. This possible interference can be influenced by several factors including the level of interfering substance in plasma, the assay principle and the end-point detection system, that is optical versus mechanical detection. The aim of this study was to determine frequency of HIL in patients' sample for coagulation analysis.

Methods: We randomly collected one month results from routine pediatric hemostasis laboratory which included 6806 samples. Routine coagulation tests, including prothrombine time (PT), activated partial thromboplastin time (aPTT) and fibrinogen were performed on Sysmex CS-5100 and CA-7000. All samples were analyzed on Sysmex CS-5100 and CA-7000 which works on photo optical end-point. Also some samples (extreme HIL) were analyzed on semi automatic Siemens BFTII based on mechanical end-point detection.

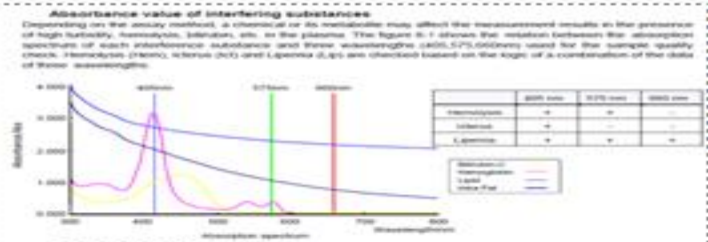


Figure 1. Relationship between absorbance and interfering substances

Figure 2. HIL Detection System

HIL Detection System

The HIL Check System requires 100 µL or more of plasma and a cuvette. Absorbance measurements at 405, 575 and 660 nm are used to classify interferences into a negative or positive judgement before ordered sample measurement begins. If the absorbance is greater than any of the criteria, the flag is displayed on job list and browser based on the setting of the system.

Table 1. HIL interference limits for some coagulation tests (Siemens reference guide document)

Test	Methodologies	Hemolysis (Hemoglobin)	Lipemia (Triglyceride)	Icterus (Bilirubin)
PT (second)	Clotting-based	400 mg/dL	203 mg/dL	48 mg/dL
APTT (second)	Clotting-based	200 mg/dL	288 mg/dL	24 mg/dL
Fibrinogen (mg/dL)	Clotting-based	400 mg/dL	289 mg/dL	6 mg/dL
Thrombin Time (second)	Clotting-based	200 mg/dL	212 mg/dL	24 mg/dL
Antithrombin III (%)	Chromogenic	1000 mg/dL	326 mg/dL	60 mg/dL
D-dimer (µg/L)	Immunologic	200 mg/dL	300 mg/dL	12 mg/dL
Coagulation Factor V	Clotting-based	1000 mg/dL	1800 mg/dL	48 mg/dL
Coagulation Factor VII	Clotting-based	1000 mg/dL	1200 mg/dL	36 mg/dL

The analyzer results is given as a level quantified within ranges:
0 = low concentration
5 = high concentration

Sysmex Recommendations / Siemens Recommendation
H = 1 : 1 (slight hemolysis)
I = 2
L = 2 : 2 (lipemia between normal (< 200 mg/dL) and abnormal (>200 mg/dL) samples)

Results: The percent of hemolyzed specimens was determined as 5.4% of all of the samples. We also identified the frequency of lipemia as 0.6% and 2.2% of samples were icteric. 7 and 8 of samples were rejected due to severe lipemia and hemolysis, respectively.

Table 2. The percent of HIL indices of samples analyzed for one month

Sample Information (Reported in Joblist)	No of Observed HIL Indices	The percent of Observed HIL Indices
Ict	150	2.2
I*	687	10.1
I*Lip	31	0.45
H* I* L*	4	0.058
Hem	2	0.029
Hem I*Lip	8	0.11
H* I*	1	0.014
Hem I*	358	5.26
H* I* Lip	3	0.044

The number of samples analyzed for one month: 6806

Table 3. The levels of HIL observed in some samples tested

No of Sample Tested	Observed HIL Indices H-Check	I-Check	L-Check	Sample Information (Reported in Joblist)
2	1	0	0	Hem
8	0	2	0	Ict
11	1	*	0	Hem I*
3	1	*	1	Hem I*
1	5	*	0	Hem I*
1	3	*	0	Hem I*
1	3	*	1	Hem I*
3	2	*	0	Hem I*
33	0	*	0	I*
2	1	*	2	Hem I*Lip
2	0	*	2	I*Lip

Table 4. Displaying sample information on joblist screen and dialog box

Display Message		Cause	Corrective action countermeasure
[Joblist] screen	[Detail] dialog box		
Hem, Ict, Lip	Hemolyzed, Icteric and Lipemic sample	Sample suspected of hemolysis, icterus and lipemia, respectively	Check the reaction curve and follow judgement criteria for the institution
Vol	Defective Sample Volume	Sample suspected of inappropriate volume	
H*, I*, L*	No message	Indicates that the influence of turbidity or other substance in the sample prevented an accurate hemolysis, icterus and lipemia check, respectively	

The [Joblist] screen and the [Detail] dialog box display the result of the inhibitor check in the sample (normal mode only) and the result of the sample volume check.

Hypertriglyceridemia and hyperlipidemia were the most common causes of severe lipemia in this study. The difficulty with lipemia may be caused by the fact that lipids vary greatly in size and composition, and thus true triglyceride concentrations do not always correlate with the results from photometric interference detection. Education and intervention may be helpful in reducing frequency of severe lipemia in patient specimens.

Table 5. The comparison of CS-5100, CA-7000 and BFTII in terms of HIL interferences

Patient number	CS-5100 Reported in Joblist	CS-5100 PT	CS-5100 INR	CS-5100 Fib*	CS-5100 APTT	CA-7000 PT	CA-7000 INR	CA-7000 APTT	CA-7000 Fib*	PLASMA INDICES H	I	L	Triglyceride (mg/dL)
1	Hem*Lip (H,*,L)	13.5 13.1 (BFTII)	1.15	193	24.2 24.6 (BFTII)	12.4 13.1 (BFTII)	0.96	25.7 24.6 (BFTII)	137	124	0	84	663
2	Hem*Lip	13.8 14.0 (BFTII)	1.18	273	39.8 [illegible] (BFTII)	14.0 14.0 (BFTII)	1.09	31.8 [illegible] (BFTII)	276	63	0	132	437
3	Hem*Lip	15.9 13.4 (BFTII)	1.11	165	41.3 43.5 (BFTII)	16.6 13.4 (BFTII)	1.18	44.3 43.5 (BFTII)	118	78	1	93	456
4	I*Lip	15.3 15.2 (BFTII)	1.29	119	16.8 [illegible] (BFTII)	14.4 15.2 (BFTII)	1.12	19.2 [illegible] (BFTII)	123	18	3	144	465
5	I*Lip	11.6 11.9 (BFTII)	0.99	256	30.1 (BFTII)	11.3 11.9 (BFTII)	0.87	30.6 30.1 (BFTII)	-	22	1	192	830
6	Hem I* (H,*,0)	12.5 11.7 (BFTII)	1.07	231	24.8 26.1 (BFTII)	11.7 11.7 (BFTII)	0.91	27.7 26.1 (BFTII)	318	154	0	29	
7	Hem I* (H,*,0)	14.2 13.2 (BFTII)	1.21	441	28.4 30.4 (BFTII)	13.8 13.2 (BFTII)	1.07	33.3 30.4 (BFTII)	440	411	1	11	
8	I*	16.4 16.8 (BFTII)	1.38	140	34.3 [illegible] (BFTII)	16.7 16.8 (BFTII)	1.3	36 [illegible] (BFTII)	144	3	48	9	

Fib*: Fibrinogen

Conclusions: Plasma index improve the quality of reported results by monitoring the degree of potential interference due to lipemia (turbidity), hemolysis and icterus (bilirubin). High incidence of icterus is belonge to pediatric samples and the recommendation of Sysmex (2 mg/dL). The Sysmex CS-5100 system's ability to provide HIL detection without additional reagents prevents extra costs and postanalytical delays. Although CS-5100 analyser HIL works as sample-specific, test-specific serum index were used to alert the operator and clinician to reject a result where necessary. The best solution for HIL is to recollect the sample; however that isn't always possible. Many laboratories report results and alert clinicians to interpret the results in the presence of HIL. These three coagulation analyzers can be used interchangeably when needed. Using the HIL check, the analyzer provides the authorized technician with a fast and objective preanalytical sample check. However, some limitation were evident in the

References
1. Bonini et al. [illegible] Preanalytical and postanalytical variables: the leading causes of diagnostic error in [illegible] Semin Thromb Hemost. 2005; 31: 612-634.
2. Woegel C. et al. HIL interferences on three hematology analyzers and contribution of a preanalytical module for routine coagulation assays. Clin Lab [illegible]
3. Lippi G. et al. Interference in coagulation testing: focus on spurious hemolysis, icterus and lipemia. Semin Thromb Hemost. 2013; 39: 258-266.
4. Lippi G. et al. Technical evaluation of the use of preanalytical modules in a laboratory. ACL TOP [illegible]


ACL TOP 700

STA Max Generation

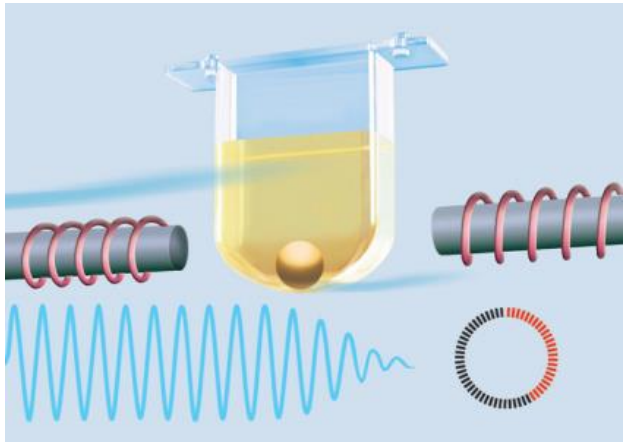
STA-R Max 2

- 360 test/saat
- 3 Prob
- 1000 küvet yükleme
- 215 numune yükleme
- Kapak delme
- Barkodlu yükleme
- Otomatik dilüsyonlar
- 70 reaktif pozisyonu
  - 15 karıştırıcılı
- Barkod kalibrasyonu
  - PT
  - Fibrinojen
- Rack ile numune
- Barkod döndürme

STA Compact Max 3

- 150 test/saat
- 3 Problu
- 1000 küvet yükleme
- 96 numune yükleme
- Kapak delme
- Barkod ile yükleme
- Otomatik dilüyonlar
- 45 reaktif pozisyon
  - 5 karıştırıcılı
- Barkod kalibriasyon
  - PT
  - Fibrinojen
- Çekmece ile yükleme

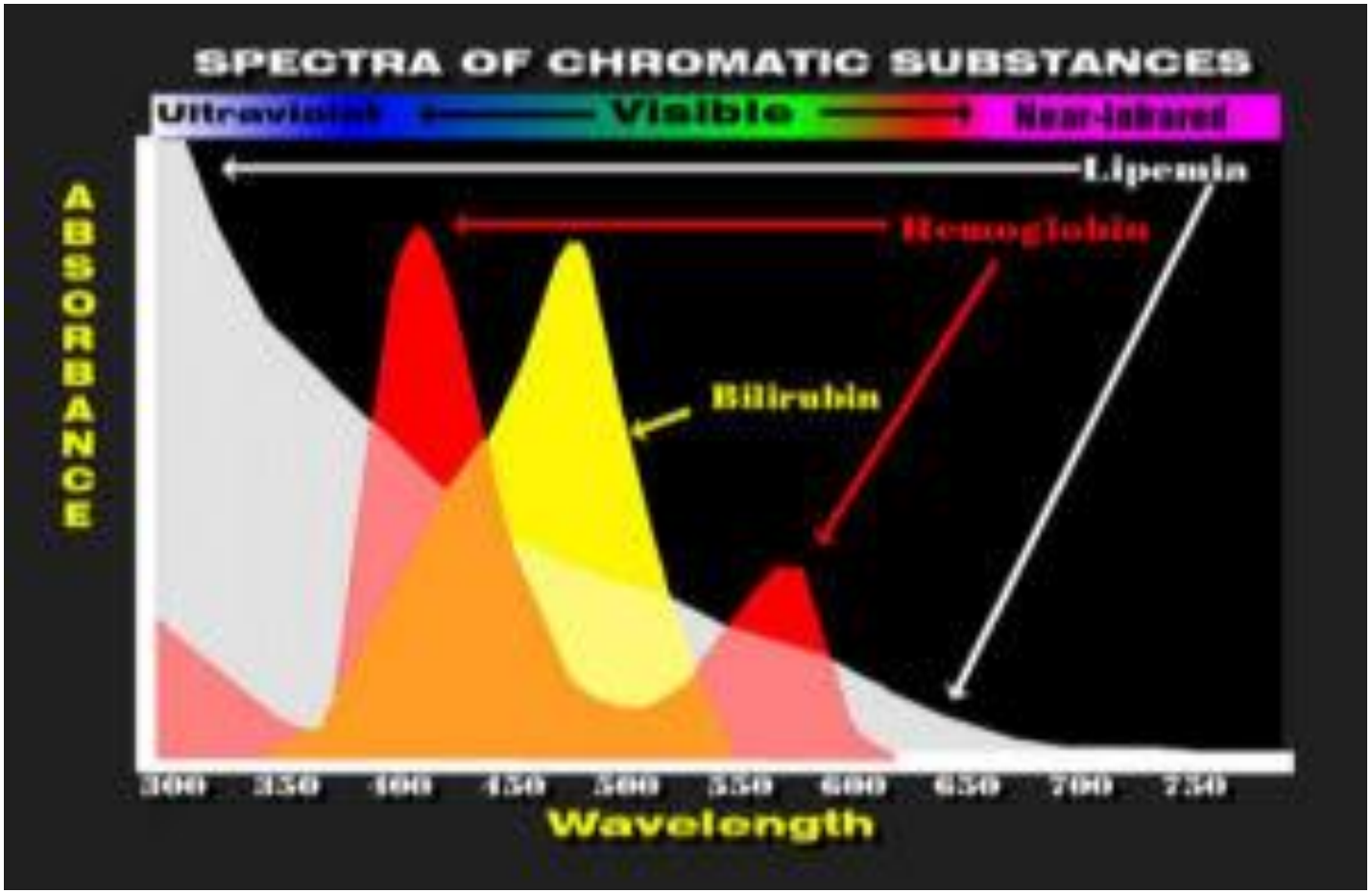
VBDS (Viscosity Based Detection System)



- Patentli Stago yöntemi
  - Mekanik Ölçüm
  - Preanalitik değişkenlerden etkilenmiyor

platelet counts of at least up to 200 x 10^9/L (200,000/µL).[13,14] Samples that have visible hemolysis should not be used because of possible clotting factor activation and end point measurement interference. Some current instruments using an optical detector may have problems with end point determinations on samples that are icteric, lipemic, or contain substances that interfere with light transmission. Alternative methods (e.g., mechanical/electromechanical) should be considered.

Optik Interferanslar



Effect of Hemolysis on Coagulation Testing

Release of ADP and other procoagulant factors

Platelet activation

Activation of coagulation cascade

Release of procoagulant factors: patient dependent

Hemoliz

Hemoglobin interferansı	Tip	Max Generation	CS Series	ACL TOP 50 Series
PT	Artificial	**5 360 mg/dL**	200 mg/dL (1) 1 000 mg/dL (1)	500 mg/dL
APTT	Artificial	**5 360 mg/dL**	200 mg/dL (1) 800 mg/dL(1)	500 mg/dL
Fib	Artificial	**5 360 mg/dL**	400 mg/dL	375 mg/dL
Kaynak		HIL study S. Kitchen (2015)	CS-5100 Reference Guide ISTH Poster S. Kitchen (2015)	Reaktif insertleri

Stago en iyi çözüm

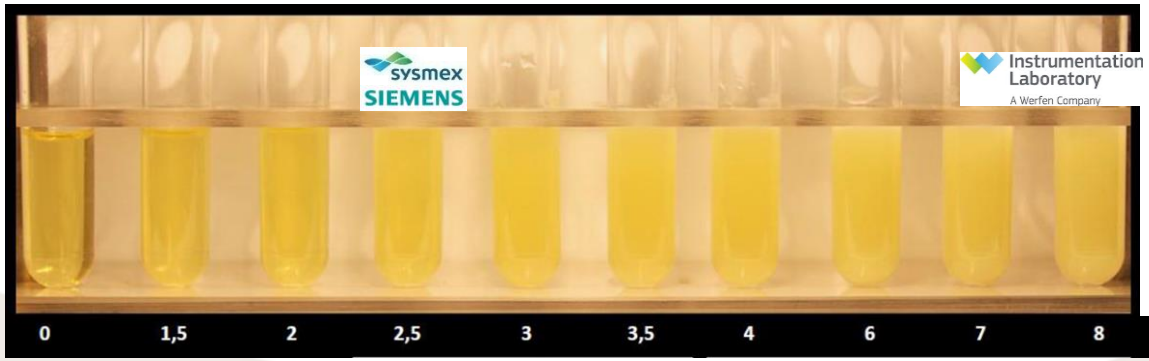
... 5 000

(1) Kullanılan reaktife göre

A Stago Group Company

Lipemik

Trigliserid Interferansı	Max Generation	CS Series	ACL TOP 50 Series
PT	**1300 mg/dL**	203 mg/dL	1000 mg/dL
APTT	**1300 mg/dL**	202-288 mg/dL	1000 mg/dL
Fib	**1300 mg/dL**	289 mg/dL	750 mg/dL
Kaynak	HIL study S. Kitchen (2015)	CS-5100 Reference Guide	Reaktif insertleri



Stago
Kesinlikle en iyi çözüm

ALBIO
A Stago Group Company

clotting (optical and optomechanical), chromogenic, immunologic, high-sensitivity luminescence based immunoassay (LOCI) technology, and platelet aggregation testing

Pre-analytical Check of Sample Integrity (PSI check)

SIEMENS Healthineers

Checking samples for proper fill volume

The sample integrity is checked prior to processing orders. PSI™ check evaluates the actual sample tube fill as well as potential interferences (HIL) since both can have an impact on patient results.

In case under or over filling is detected, the red PSI check symbol appears next to the sample ID. The following proper-fill check results can be displayed:

- The sample tube is under filled or over filled, but the sample can be measured. A symbol is displayed in the dialog **Jobs**, a flag is displayed in the dialog **Jobs > Sample result info**, and a symbol is displayed in the column **Level** in the dialog **Jobs > Sample info**.

Pre-analytical Check of Sample Integrity (PSI check)

SIEMENS Healthineers

Measurement Principle

The HIL reader performs an initial check of samples relative to hemolysis, icterus, and lipemia.

A photometer in the HIL reader checks the presence and the type (H, I, or L) of interference in the sample.

- H = hemoglobin resulting from lysis of red blood cells
- I = icterus resulting from endogenous bilirubin
- L = lipemia or turbidity caused by insoluble lipids

The following results can be displayed:

- PSI • Above sample-specific H, I or L warning level
- PSI • Above assay-specific H, I or L warning level. The sample's hemolytic, icteric or lipemic index is higher than or equal to the assay-specific warning level
- HIL • HIL was not measured or is deactivated. A visual check for interfering substances is recommended

cobas t 711 ve t 511 koagülasyon analizörleri

Bir bakışta

Figure 41.1. Advantages and disadvantages of detection methods in defining parameters

Method	Advantages	Disadvantages
Mechanical	• No interference due to physical characteristics such as lipemia or hemolysis • May use small sample volumes • Some can analyse whole blood for some tests, removing the need for centrifugation	• Impossible to observe graphics of clot formation • May present problems of endopoint detection in some samples with low fibrinogen
Photo-optic	• Possibility of graphics on clot formation • Optical checks for hemolysis/lipemia/icterus on some optical systems	• Interference due to lipemia, hemolysis, hyperbilirubinemia, or protein increase on some systems • Some systems may present difficulties with clot detection when using some completely transparent reagents • Very short coagulation periods may go undetected owing to delay prior to initiation of monitoring

Nephelometric	• Can measure antigen-antibody reactions in proteins present in very small amounts	• Limits number of available tests • Cost of reagents
Chromogenic	• Fully specific assays may be easier • Additional parameters not suitable for measurement by clot detection may be possible • Increases the repertoire of possible tests • Possible improvements in precision compared to clot based analyses	• Limited by the instrument's wavelength • Requires large test volumes for positive cost-benefit ratio • Cost of instrument and reagents
Immunological	• Can automate time-consuming, manual methods • Increases the number of possible tests	• Limited number of tests available • Cost of instruments • Cost of reagents

İki farklı markanın PT kiti

Limitations of the Procedure

Normal samples spiked with heparin concentrations exceeding 0.6 U/mL produced abnormal results. However, Thromborel® S Reagent may be used to monitor the administration of overlapping dosages of heparin and oral anticoagulants. In thrombolysis therapy, derived fibrinogen and fibrinogen determination according to Clauss may deviate and should be considered in therapy control. Inhibitors of the Lupus type anticoagulant can influence prothrombin time and lead to INRs that do not accurately reflect the true level of anticoagulation[5]. The choice of anticoagulant (i.e. oxalate instead of citrate) and the condition of the specimen (e.g hemolyzed, lipemic, parenteral feeding, etc.) may affect results. The latter is particularly true for PT measurements done with optical instruments. Hirudin or other direct thrombin inhibitors in therapeutic dose result in prolonged prothrombin times[6-8].

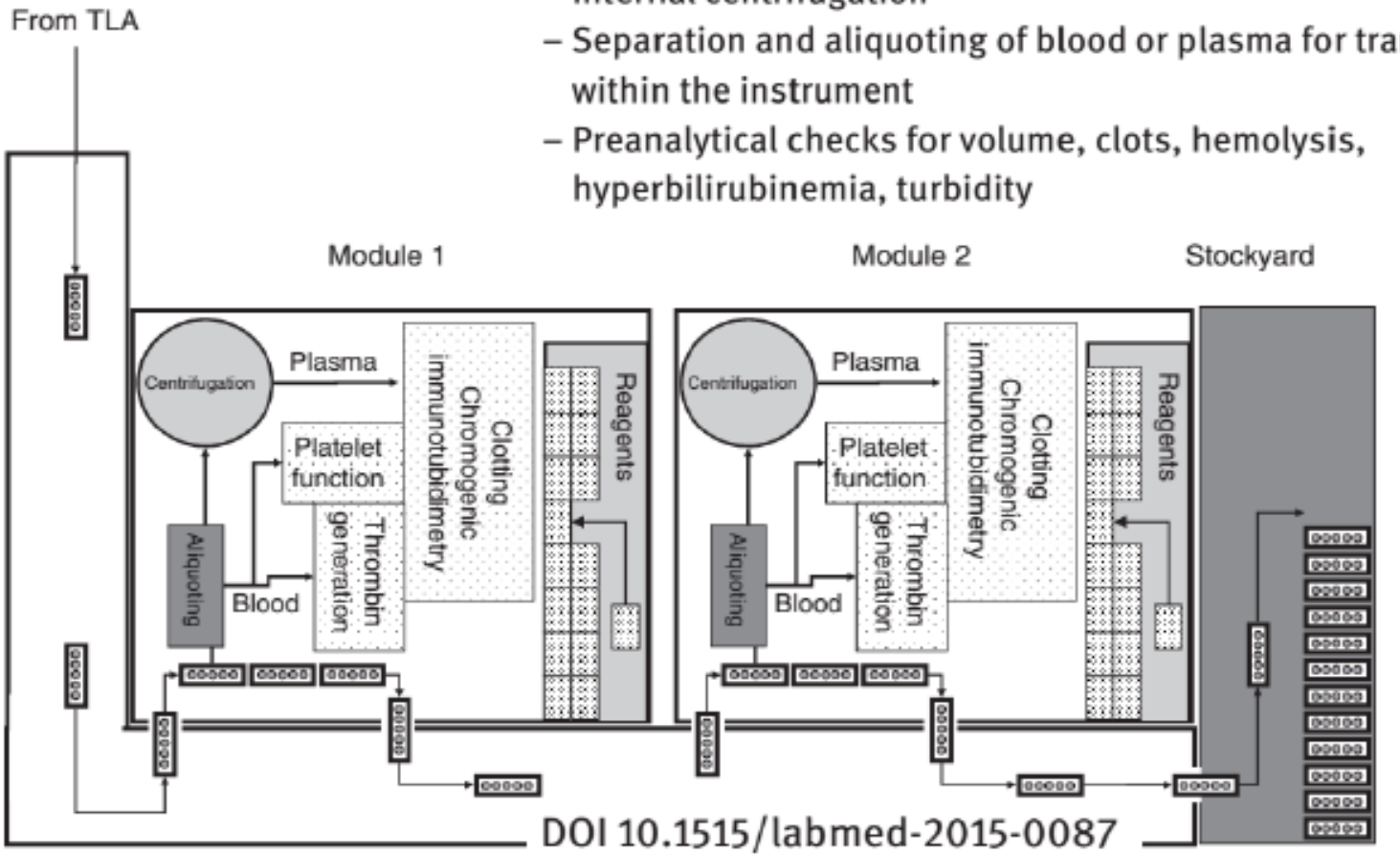
11/ LIMITATIONS

- **Sample**
  The slightest coagulation (micro-clots) will induce considerable shortening of the times measured (autocatalytic activation of all the factors) whereas extensive coagulation will prolong the clotting times because of consumption of factors and fibrinogen.
  Do not keep plasmas at 2-8 °C because in this temperature range the factor VII may be activated by the kallikrein system (2).
- **Anticoagulant**
  Maintain the correct anticoagulant/blood sample volume ratio of 1:9. If there is any considerable variation in hematocrit, modify the quantity of anticoagulant accordingly.
- **Heparins**
  The STA® - Néoplastine® CI Plus test is insensitive to unfractionated heparin levels up to 1 IU/ml and to low molecular weight heparin levels up to 1.5 anti-Xa IU/ml.

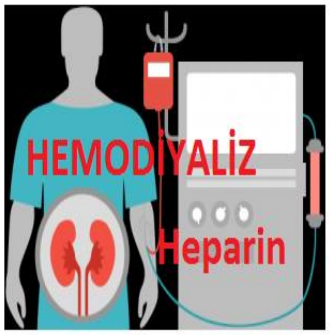
Giuseppe Lippi*, Chiara Bovo and Emmanuel J. Favaloro

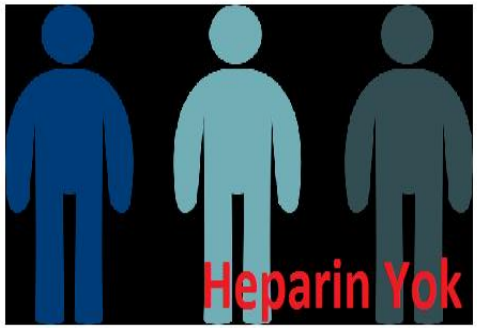
Reflections on the next generation of hemostasis instrumentation. A glimpse into the future?

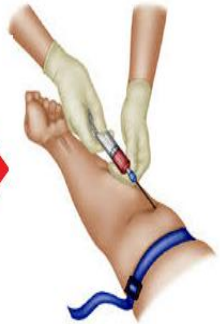
Preanalytical sample management
- Multiple sample types management
- Internal centrifugation
- Separation and aliquoting of blood or plasma for transportation within the instrument
- Preanalytical checks for volume, clots, hemolysis, hyperbilirubinemia, turbidity



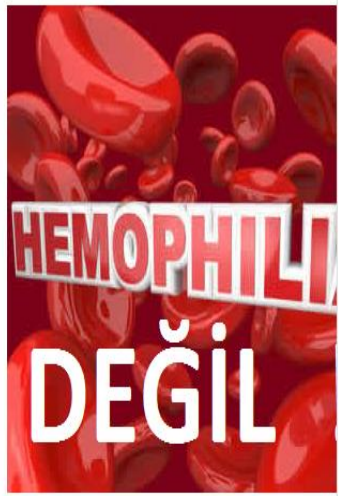
DOI 10.1515/labmed-2015-0087

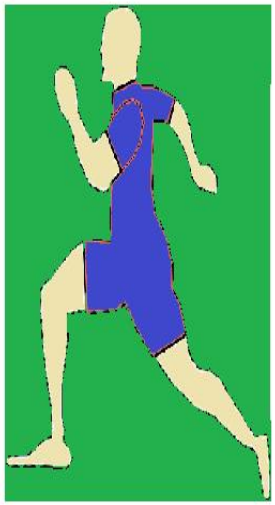

HEMODİYALİZ
Heparin

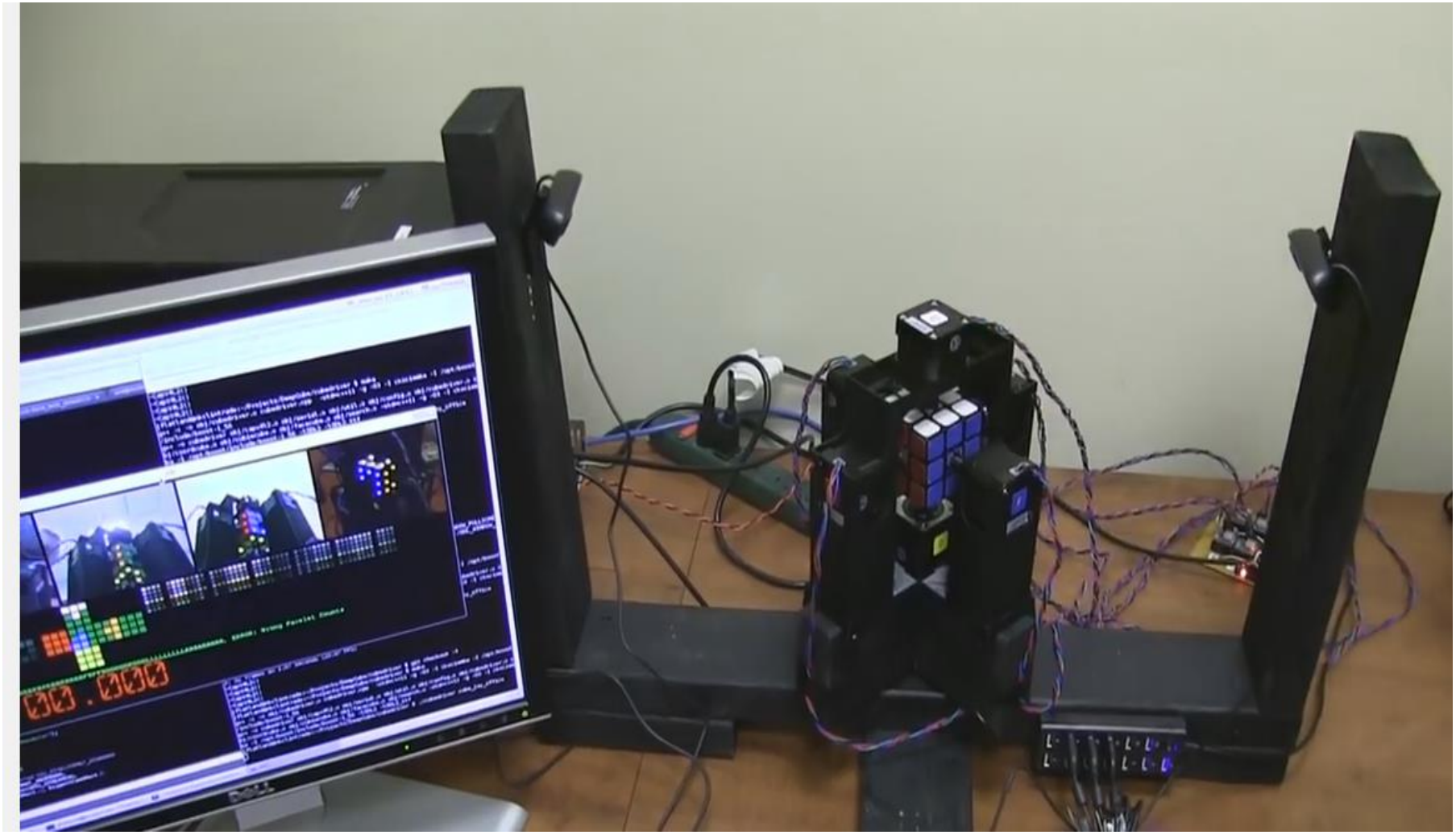

Heparin Yok !




Coagulometer


HEMOPHILI
DEĞİL





0.1.196 saniye

Pre-analytical error might lead to inaccurate result and compromise patient safety!

Int J Lab Hematol. 2015 Feb;37(1):50-5. doi: 10.1111/ijlh.12230. Epub 2014 Apr 8.

The order of draw: much ado about nothing?

Indevuyst C[1], Schuermans W, Bailleul E, Meeus P.

Author information

Abstract

INTRODUCTION: The 'order of draw' has been advocated since 1982 to reduce the risk of cross-contaminating blood tubes with additives from a previously filled tube.

METHODS: We studied 193 patients receiving oral anticoagulation. Multiple tubes were collected in a specific order using the Sarstedt Safety Monovette System. We evaluated the effect of the 'order of draw' on the prothrombin time/international normalized ratio (PT/INR) and the activated partial thromboplastin time (APTT) when the citrate tube is drawn as the first tube, second tube or after a heparin, EDTA or serum tube with clot activator.

RESULTS: There was no statistically significant influence on the PT/INR. The same applies for the APTT measured on a citrate tube drawn after a heparin tube. There was a small, but statistically significant bias on the APTT when the citrate tube was drawn as the first tube, after an EDTA tube or after a serum tube with clot activator. We consider this bias (max. 0.2 s) as not clinically significant.

CONCLUSION: The order of draw has no significant influence on the PT/INR and APTT when measured on a Sarstedt citrate tube filled without the use of a discard tube or after a heparin, EDTA or serum tube with clot activator.

Biochem Med (Zagreb). 2017 Feb 15;27(1):217-224. doi: 10.11613/BM.2017.023.

Pneumatic tube system transport does not alter platelet function in optical and whole blood aggregometry, prothrombin time, activated partial thromboplastin time, platelet count and fibrinogen in patients on anti-platelet drug therapy.

Enko D[1], Mangge H[2], Münch A[3], Niedrist T[2], Mahla E[4], Metzler H[3], Prüller F[5].

⊕ Author information

Son Söz

Everything begins with the Sample

1st Things 1st

Of-line preanalitik sistemler hem istenen özellikteki santrifuj ve plazma indeksini vermesi ve örnek hacmini belirlemesi nedeniyle bu özellikleri taşımayan koagülasyon cihazların açığını kapatabilir, en doğrusu preanalitik özellik yönünden cihaz ve reaktifleri, cihaz seçiminde ön plana çıkarmaktır.

TEŞEKKÜRLER